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Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744

(bioluminescence/regulation/autoinduction/repression/activator)

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ABSTRACT **_Escherichia coli_ that carry a recombinant plasmid bearing the _Vibrio fischeri lux_ regulon express luminescence that mimics the luminescence of _V. fischeri_. The _lux_ regulon consists of two divergently transcribed operons, the rightward operon (_luxICDABE_ genes) and the leftward operon (_luxR_ gene). The _luxR_ and _luxI_ genes and the control region separating the two operons supply the primary regulatory control over the _lux_ regulon; the regulatory mechanisms result in a dramatic increase in the rate of luciferase synthesis after induction, apparently due to a unique autoregulatory positive feedback mechanism, and in an enormous difference (>10^4) in levels of luminescence in cells before and after induction. The generally accepted model of primary regulation of bioluminescence in _V. fischeri_ involves the interaction of the product of the _luxR_ gene and _N_-(3-oxohexanoyl)homoserine lactone, the autoinducer produced by the enzyme encoded by _luxI_, the first gene of the rightward operon, with an operator sequence within the control region to stimulate transcription of the rightward operon in a positive feedback loop. We have used deletion mapping of a transcription reporter vector to determine the approximate location of the operator. By site-directed mutagenesis of the presumed operator, we have demonstrated that the 20-base-pair inverted repeat ACCTGTAGGA|TCGTA CAGGT (where the vertical line is the center of symmetry), which bears striking similarity to the recognition sequence for the pleiotropic repressor protein LexA, is the operator of the _lux_ regulon. We also found that deletion of sequences upstream of the palindrome leads to increased transcription from the rightward promoter (P_R), indicative of a cis-acting element that represses transcription in the absence of the LuxR–autoinducer complex. Modifications of the palindrome that eliminate stimulation by LuxR–autoinducer of transcription from P_R have no effect on repression by the cis-acting mechanism(s), suggesting that the palindrome is not necessary for repression of the rightward operon. Thus, it appears that the large increase in transcription upon induction of the _lux_ regulon is the result of at least two independent mechanisms, one positive and the other negative.**

Luminescence from cultures of marine bacteria, which is strongly dependent on cell density, has been a subject of inquiry for many years (1). Kempner and Hanson (2) ascribed the lag in appearance of luminescence after inoculation of a broth culture to metabolism of inhibitors in the medium. However, later, the lag in appearance of luminescence was shown to be caused by a small dialyzable molecule produced by the bacteria. Nealson and his colleagues (3, 4) called this phenomenon "autoinduction." More detailed descriptions of autoinduction led to the isolation and structural elucidation of the autoinducer of *Vibrio fischeri* (5). This substance, *N*-(3-oxo-hexanoyl)homoserine lactone, has been synthesized and shown to function in a biological assay system (5, 6). Investigation of the effect of the synthetic autoinducer on expression of luminescence from a natural isolate of *V. fischeri* deficient in autoinducer synthesis confirmed that the autoinducer is freely diffusible and effective at low concentrations (7).

The genes required for regulated bioluminescence are located in a regulon that has been cloned from *V. fischeri* strains MJ-1 and ATCC7744. Light emission from bioluminescent marine bacteria or *Escherichia coli* carrying the *lux* regulon results from the activity of the enzyme bacterial luciferase, a flavin monooxygenase (for review, see ref. 8). Luciferase is a heterodimeric enzyme; the α and β subunits are encoded by the *luxA* and *luxB* genes, respectively. The aldehyde substrate is supplied by the products of the *luxC*, *luxD*, and *luxE* genes that form a fatty acid reductase complex responsible for the reduction of myristic (tetradecanoic) acid (9). The reduced flavin for the luciferase reaction can be supplied by *E. coli* enzymes. Through an extensive series of experiments employing transposon insertion mutagenesis, mini-Mud *lac* fusions, and protein programming in minicells, Silverman and coworkers (10, 11) have delineated the structural organization of the regulon. We have determined the nucleotide sequence of the entire *lux* regulon from *V. fischeri* (T.O.B., J.H.D., R. Heckel, T. C. Johnston, and J.-W. Lin, unpublished data). Based on the genetic studies and our sequence data, the physical map of the regulon presented in Fig. 1 is now firmly established.

E. coli that carry the *lux* regulon express the luminescence genes in a fashion that mimics the regulatory pattern observed in *V. fischeri* (10). Expression of the *lux* regulon is modulated by the cAMP-binding protein (CAP)–cAMP system (12, 13), by the autoinducer–LuxR system, and possibly by other regulatory processes superimposed upon these primary systems, including the SOS system and the heat shock response (14).

It has been suggested (10) that the primary level of control of the *lux* regulon is a positive feedback loop in which basal transcription of *luxI* leads to accumulation of a low level of autoinducer. The diffusible autoinducer provides a mechanism by which a strong coordinated response can be achieved from a population of cells. If LuxR is present, the LuxR–autoinducer complex stimulates transcription of P_R, the promoter of the rightward operon. Such stimulation would lead to increased levels of LuxI and autoinducer, which would further activate LuxR. Since the autoinducer is freely diffusible, the induction of one cell can lead directly to the induction of others. A positive feedback circuit can generate a large and rapid response to a small initial stimulus, but it is seldom employed in cellular systems because, once it has been initiated, it is difficult to control. Viral systems, however, often employ positive feedback after host-cell stability is no longer required. To prevent the excessive depletion of

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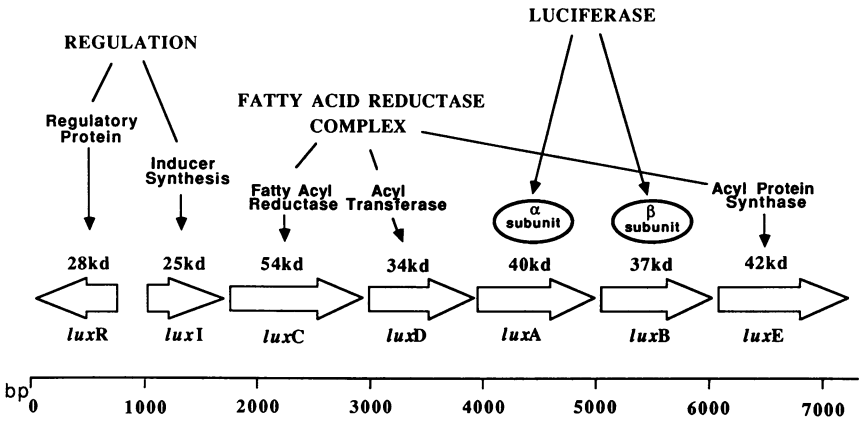

FIG. 1. Physical map of the *lux* regulon of *V. fischeri* strain ATCC7744. kd, kDa.

a cell's resources by a positive feedback response, it is important to have a strong stopping mechanism and to apply this mechanism before the response has drained cellular reserves. It has been suggested that the LuxR–autoinducer complex functions at either the translational (15) or transcriptional (13) level in an autoregulatory fashion to slow production of LuxR, thereby limiting the positive feedback loop.

To understand the network of events that regulate bacterial bioluminescence, we have investigated the location and nature of the operator region of the *lux* regulon by first constructing deletions of the leftward operon extending into the control region. By supplying synthetic autoinducer and functional LuxR in trans, we tested the ability of the remaining sequences to interact with LuxR–autoinducer to stimulate transcription of the rightward operon. Site-directed mutagenesis confirmed that the operator is the 20-base-pair (bp) inverted repeat in the control region (16). A preliminary report of this work has been presented.‡

MATERIALS AND METHODS

Materials. Primers for DNA sequencing were custom synthesized using an Applied Biosystems model 380B DNA synthesizer. Autoinducer, which was synthesized by the procedure of Eberhard *et al.* (5), was diluted 1:10,000 (wt/vol) in water and stored at 4°C. Cultures were supplemented with autoinducer by a 1:1000 dilution of the stock solution into culture medium. *E. coli* strain TB1 (ref. 17; *hsdR*$^-$, Δ*lac pro*) was used for all expression experiments. The P_R transcription reporter vector, pJHD500 (6.7 kbp, Fig. 2), was constructed in a multistep process by combining 3.2 kbp of pBR322 DNA bearing the colE1 origin and the *bla* ampicillin-resistance gene, 2.3 kbp of *Vibrio harveyi luxAB* DNA, and 1.2 kbp of *V. fischeri* DNA containing the intact *luxR* gene, a portion of the *luxI* gene, and the regulatory region.

Plasmids pJHD504 and -505 were derived from pJHD501, a variant of pJHD500 in which a single-base change created a unique *Kpn* I–*Asp*718 site immediately upstream of the 20-bp inverted repeat in the *V. fischeri* control region. Plasmids pJHD504 and -505 were constructed from pJHD501 by cutting with *Ava* I and *Asp*718 or *Kpn* I, respectively. The DNA was treated with Klenow fragment of DNA polymerase I and dNTPs, which filled-in the *Ava* I and *Asp*718 ends and blunted the 3′ ends generated by *Kpn* I with its 3′ → 5′ exonuclease activity. The fragments were then religated and used to transform *E. coli* strain TB1 to ampicillin resistance. Functional LuxR was supplied on the trans-complementing plasmid pAC102, a pACYC177-derived plasmid conferring kanamycin resistance and containing an intact *luxR* gene.

‡Baldwin, T. O., Devine, J. H., Heckel, R. C., Lin, J.-W., & Shadel, G. S., Fifth International Symposium on Bioluminescence and Chemiluminescence, Sept. 25–29, 1988, Florence, Italy.

Site-Directed Mutagenesis. Site-directed mutagenesis was accomplished by a slight variation of the method of Kunkel *et al.* (18). Single-stranded uracil-containing DNA was used as template for the mutagenesis reactions; template was prepared from phagemid-infected cells rather than from M13 as described by Kunkel *et al.* (18).

Cell Growth and Measurement of Bioluminescence. Growth measurements were initiated by 1:200 dilution of a fresh overnight culture into 40 ml of Luria broth (LB) containing ampicillin (100 mg/liter) in 250-ml flasks. The cultures were shaken at 100 rpm in a waterbath shaker controlled at 27–29°C. At various times, 2-ml aliquots were withdrawn and used immediately for determination of the absorbance at 600 nm. Luciferase activity in 1 ml of each sample was measured using a photomultiplier photometer (19) for which 1 light unit (Lu) = 9.8×10^9 quanta per sec referenced to the light standard of Hastings and Weber (20). The substrate used was a freshly prepared suspension of 10 μl of decanal sonicated in 10 ml of LB. Assays *in vivo* were carried out by rapid injection of 1 ml of the substrate suspension into 1 ml of cell culture and measuring the peak intensity of the emitted light.

Determination of Nucleotide Sequence. Plasmid pHK705 (21), containing the regulatory functions of the *lux* regulon from *V. fischeri* strain MJ-1, was the generous gift of Peter Greenberg (University of Iowa). Double-stranded template DNA was prepared from overnight bacterial cultures using the alkaline lysis method (22) followed by treatment of the DNA pellet with RNase A and precipitation with PEG 8000. The

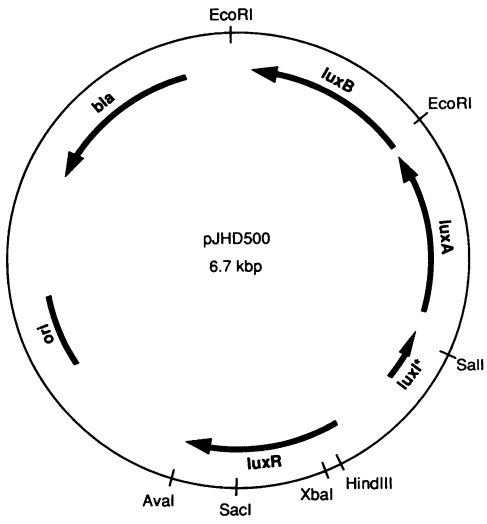

FIG. 2. Plasmid map of the transcriptional reporter, pJHD500. Transcription from the rightward operon can be monitored with ease and sensitivity by measuring the light produced by cells bearing this or related plasmids upon addition of exogenous decanal.

Table 1. Primers used to sequence *luxR* from *V. fischeri* MJ-1

Message equivalent primers	Primer sequence	Location	Message complementary primers	Primer sequence	Location
R3	5′-AATGCCGACGACACTTACAG-3′	13–32	R8	5′-CGGCATTTATGTCTTTCATACCC-3′	19–(−)4
R4	5′-ATCTGATATTTCAATTCTAG-3′	159–158	R11	5′-GATTTCATGGAAATGTAT-3′	843–825*
R5	5′-GCTGTAAATAAAAAATCTCC-3′	301–320	R12	5′-GGGCAATCAATTGCTCCTG-3′	737–718
R6	5′-GATAGTTTATTTTTACATGC-3′	442–461	R13	5′-CTCTTTTGGTTAAATCGT-3′	559–542
R7	5′-CGAAGGAAAGAGTCTTGGG-3′	585–604	R14	5′-GGGAAACTAAACCCAGTG-3′	374–357
M13	Reverse primer		R15	5′-GCCTCCATTTTTTAGGGT-3′	202–185

Indicated primers were used to determine the sequence of both strands of the *luxR* gene of *V. fischeri* strain MJ-1 and to confirm the published sequence of the gene from strain ATCC7744 (16). The indicated nucleotide positions refer to the numbering system of Fig. 3 in which position 1 is the adenine of the second ATG. Negative numbers (primer R8) refer to positions on the 5′ side of position 1.
*The indicated location of primer R11 is beyond the published sequence of the *lux* regulatory region (16). The indicated positions are relative to the sequence presented in Fig. 3, as are the other primer locations.

purified plasmid DNA was then denatured with 0.2 M NaOH (23) and used as a template for sequencing by the dideoxynucleotide chain-termination method using modified T7 DNA polymerase (Sequenase) (24) and the primers listed in Table 1.

RESULTS

Nucleotide sequences of the regulatory regions of the *lux* regulons from *V. fischeri* strains ATCC7744 and MJ-1 have been reported (16, 25); in our report of the ATCC7744 sequence, we pointed out the existence of a 20-bp palindrome in a position that suggested a protein-binding role, potentially functioning in the regulation of the *lux* regulon. The reported MJ-1 sequence (25) within the control region was nearly identical to the ATCC7744 sequence, but there were major differences within the *luxR* coding region. Since major differences between the strains were not expected, we have determined the sequence of both strands of the *luxR* coding region from plasmid pHK705 (21). The correct sequence[§] of the *luxR* gene from *V. fischeri* MJ-1 is presented in Fig. 3 and aligned with the published sequence (16) of the same region

[§]These sequences have been deposited in the GenBank data base [accession nos. M25751 (for the MJ1 sequence) and M25752 (for the ATTC7744 sequence)].

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                                                                30                                                  60
                                M?  G?  M   K   N   I   N   A   D   D   T   Y   R   I   I   N   K   I   K   A   C   R   S   N
MJ-1                    §AGAG ATG GGT ATG AAA aAC ATA AAT GCC GAC GAC ACa TAC AGA ATA ATT AAT AAA ATT AAA GCT TGT AGA AGC AAT
7744 GTAAGGATAAAGAG ATG GGT ATG AAA gAC ATA AAT GCC GAC GAC ACt TAC AGA ATA ATT AAT AAA ATT AAA GCT TGT AGA AGC AAT
                                    D
                                                90                                                  120                                                 150
 N   D   I   N   Q   C   L   S   D   M   T   K   M   V   H   C   E   Y   Y   L   L   A   I   I   Y   P   H   S   M
AAT GAT ATT AAT CAA TGC TTA TCT GAT ATG ACT AAA ATG GTA CAT TGT GAA TAT TAT TTA CTC GCG ATC ATT TAT CCT CAT TCt ATG
AAT GAT ATT AAT CAA TGC TTA TCT GAT ATG ACT AAA ATG GTA CAT TGT GAA TAT TAT TTA CTC GCG ATC ATT TAT CCT CAT TCc ATG

                                        180                                                 210                                                 240
 V   K   S   D   I   S   I   L   D   N   Y   P   K   K   W   R   Q   Y   Y   D   D   A   N   L   I   K   Y   D   P
GTT AAA TCT GAT ATT TCA ATT CTA GAT AAT TAC CCT AAA AAA TGG AGG CAA TAT TAT GAT GAC GCT AAT TTA ATA AAA TAT GAT CCT
GTT AAA TCT GAT ATT TCA ATT CTA GAT AAT TAC CCT AAA AAA TGG AGG CAA TAT TAT GAT GAC GCT AAT TTA ATA AAA TAT GAT CCT

                                            270                                                 300
 I   V   D   Y   S   N   S   N   H   S   P   I   N   W   N   I   F   E   N   N   A   V   N   K   K   S   P   N   V
ATA GTA GAT TAT TCT AAC TCC AAT CAT TCA CCA ATT AAT TGG AAT ATA TTT GAA AAC AAT GCT GTA AAT AAA AAA TCT CCA AAT GTA
ATA GTA GAT TAT TCT AAC TCC AAT CAT TCA CCA ATT AAT TGG AAT ATA TTT GAA AAC AAT GCT GTA AAT AAA AAA TCT CCA AAT GTA

                                                360                                                 390
 I   K   E   A   K   T   S   G   L   I   T   G   F   S   F   P   I   H   T   A   N   N   G   F   G   M   L   S   F
ATT AAA GAA GCG AAA aCA TCA GGT CTT ATC ACT GGG TTT AGT TTC CCT ATT CAT ACg GCT AAc AAT GGC TTC GGA ATG CTT AGT TTT
ATT AAA GAA GCG AAA tCA TCA GGT CTT ATC ACT GGG TTT AGT TTC CCT ATT CAT ACt GCT AAt AAT GGC TTC GGA ATG CTT AGT TTT
                    S
    420                                             450                                                 480
 A   H   S   E   K   D   N   Y   I   D   S   L   F   L   H   A   C   M   N   I   P   L   I   V   P   S   L   V   D
GCA CAT TCA GAa AAA GAC AAC TAT ATA GAT AGT TTA TTT TTA CAT GCg TGT ATG AAC ATA CCA TTA ATT GTT CCT TCT CTA GTT GAT
GCA CAT TCA GAg AAA GAC AAC TAT ATA GAT AGT TTA TTT TTA CAT GCa TGT ATG AAC ATA CCA TTA ATT GTT CCT TCT CTA GTT GAT

        510                                             540                                                 570
 N   Y   R   K   I   N   I   A   N   N   K   S   N   N   D   L   T   K   R   E   K   E   C   L   A   W   A   C   E
AAT TAT CGA AAA ATA AAT ATA GCA AAT AAT AAA TCA AAC AAC GAT TTA ACC AAA AGA GAA AAA GAA TGT TTA GCG TGG GCA TGC GAA
AAT TAT CGA AAA ATA AAT ATA GCA AAT AAT AAA TCA AAC AAC GAT TTA ACC AAA AGA GAA AAA GAA TGT TTA GCG TGG GCA TGC GAA

            600                                             630                                                 660
 G   K   S   S   W   D   I   S   K   I   L   G   C   S   E   R   T   V   T   F   H   L   T   N   A   Q   M   K   L
GGA AAa AGC TCT TGG GAT ATT TCA AAA ATA TTA GGC TGC AGT gAG CGT ACt GTC ACT TTC CAT TTA ACC AAT GCG CAA ATG AAA CTC
GGA AAg AGC TCT TGG GAT ATT TCA AAA ATA TTA GGC TGC AGT aAG CGT ACg GTC ACT TTC CAT TTA ACC AAT GCG CAA ATG AAA CTC
                                                        K
                690                                             720                                                 750
 N   T   T   N   R   C   Q   S   I   S   K   A   I   L   T   G   A   I   D   C   P   Y   F   K   N
AAT ACA ACA AAC CGC TGC CAA AGT ATT TCT AAA GCA ATT TTA ACA GGA GCA ATT GAT TGC CCA TAC TTT AAA AaT TAA
AAT ACA ACA AAC CGC TGC CAA AGT ATT TCT AAA GCA ATT TTA ACA GGA GCA ATT GAT TGC CCA TAC TTT AAA AgT TAA
                                                                                                        S
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FIG. 3. Comparison of the nucleotide and encoded amino acid sequences of the *luxR* genes of *V. fischeri* strains MJ-1 (upper lines) and ATCC7744 (lower lines). The position marked § in the MJ-1 sequence indicates the limit of the deletion used in the construction of plasmid pHK705 (ref. 21; see text). The amino-terminal sequence of Met-Gly- is marked "?" due to the uncertainty regarding the correct translational start for this protein (see text).

from the ATCC7744 strain. Several points are worthy of mention. (*i*) Only 12 replacements were observed in the corrected MJ-1 *luxR* gene sequence and only 4 of those resulted in amino acid changes. (*ii*) Both strains have an in-frame ATG prior to the designated start of the protein. The start of the coding region of LuxR was proposed on the basis of the amino acid sequence of the overproduced protein reported by Kaplan and Greenberg (21). pHK724, the plasmid used to overproduce LuxR, was constructed by deleting sequences upstream of *luxR*, a process that may have altered or removed the original ribosomal binding signals. The possibility remains that the correct LuxR is initiated at the first ATG.

Location of the Operator. We have suggested that the 20-bp inverted repeat within the *lux* regulatory region, ACCTGTAGGA|TCGTACAGGT (where the vertical line is the center of symmetry), might constitute a protein binding site and thereby serve a regulatory role (16). To test this suggestion, we have constructed deletions of the *luxR* gene up to and including portions of the palindrome. The effects of these deletions and the ability of functional LuxR added in trans to stimulate transcription of the rightward operon were evaluated by measuring the activity of the *V. harveyi* luciferase produced from the transcriptional fusions. The effects of the deletions on the ability of LuxR–autoinducer to stimulate transcription from the rightward promoter are presented in Fig. 4. Both pJHD504 and -505 displayed increased luciferase synthesis upon addition of autoinducer. The palindrome was intact in the former and lacked the AC sequence from the 5′ end in the latter. The construct with the 2-base abbreviated palindrome, pJHD505, suffered a slight but significant (≈30%) decrease in stimulation of transcription from P_R by LuxR–autoinducer. The data presented in Fig. 4 demonstrate that sequences upstream of the 20-bp palindrome are not required for autoinducer-dependent transcriptional activation. Interestingly, the uninduced levels of luciferase synthesis from both plasmids were elevated at least 10-fold above the uninduced levels for pJHD500, suggesting either that upstream sequences have a strong repressive effect on transcription from P_R or that there is a promoter within the pBR322 sequences that becomes apparent as *luxR* sequences are deleted. To discriminate between the two possibilities, the entire *V. fischeri* DNA insert was removed from the reporter vector. Cells carrying the resulting plasmid expressed luciferase activity at a level below the basal level of pJHD500, demonstrating that there is no strong promoter downstream of *luxR* in the reporter plasmid. It thus appears that upstream sequences in *luxR* have a repressive effect, in cis, on transcription from P_R.

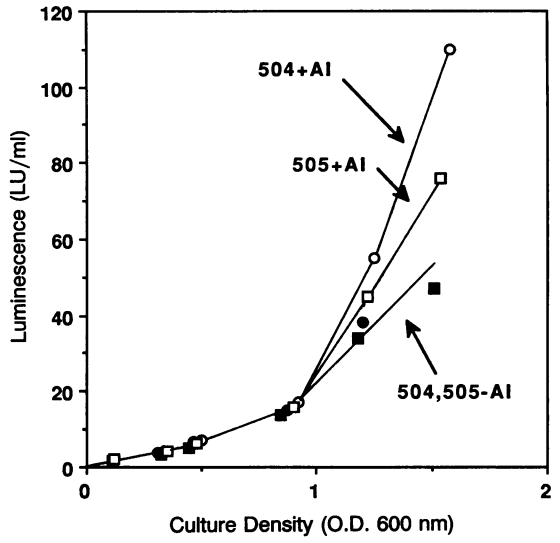

FIG. 4. In trans complementation of plasmids pJHD504 and pJHD505. Bioluminescence *in vivo* of *E. coli* TB1 cells bearing plasmid pAC102 and the deletion plasmids pJHD504 (circles) or pJHD505 (squares) at 28°C in LB containing carbenicillin (100 mg/liter) and kanamycin (25 mg/liter) is shown. Functional LuxR was added in trans by cotransformation with plasmid pAC102 that was constructed by insertion of the *luxR/luxI** sequences (see Fig. 2) into the polylinker region of plasmid pAC9. Plasmid pAC9 was constructed by insertion of the *Hae* II fragment containing the polylinker and *lacZ′* regions of pUC9 into pACYC177 from which the ampicillin-resistance marker had been deleted through removal of a *Pst* I–*Hae* II fragment. Open symbols, cultures grown with autoinducer (AI) added at an OD_{600} of 0.1; solid symbols, cultures grown without autoinducer. LU, light units.

To further evaluate the hypothesis that the 20-bp palindrome is the operator of the *lux* regulon, the central 12 bases of the palindrome were removed from the reporter plasmid by site-directed mutagenesis to generate plasmid pJHD506. Basal transcription from P_R was repressed in this construction to approximately the level seen in pJHD500, but the system no longer responded to LuxR–autoinducer (Fig. 5). The fact that the basal transcription from P_R was not altered by deletion of the central 12 bp of the palindrome demonstrated that the palindrome is not directly involved in repression of transcription from P_R by upstream sequences (see Fig. 4).

Construction and Properties of Operator Point Mutants. As a more subtle probe of the role of the base sequence in the 20-bp palindrome, we generated two point mutants. The guanine at position 5 was changed to a cytosine (op5GC) and the cytosine at position 3 was changed to a thymine (op3CT) so that the mutant palindromes were ACCTCTAGGA|TCGTACAGGT (op5GC) and ACTTGTAGGA|TCGTACAGGT (op3CT). Other than these single-base changes, the constructs were identical to pJHD500. Transcriptional activity from P_R was monitored as described above (Fig. 6). Mutant op3CT appeared to be effectively unresponsive to autoinducer, whereas mutant op5GC showed an ≈3-fold stimulation of transcription in response to the autoinducer; transcription from P_R in pJHD500, the wild-type construct, was stimulated ≈100-fold by LuxR–autoinducer. These results support the hypothesis that the 20-bp palindrome comprises a major portion of the LuxR–autoinducer binding site.

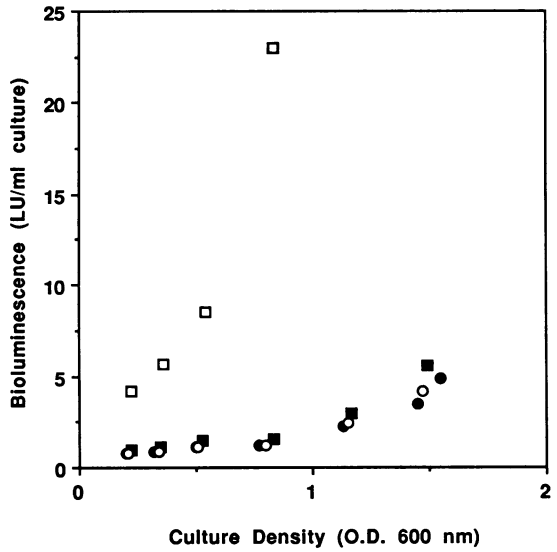

FIG. 5. Effect of deleting 12 bp within the proposed operator. Cultures of *E. coli* TB1 bearing pJHD500 (squares) or pJHD506 (circles) were grown at 30°C in LB containing ampicillin (100 mg/liter). Open symbols, cultures that contain autoinducer; solid symbols, control cultures. LU, light units.

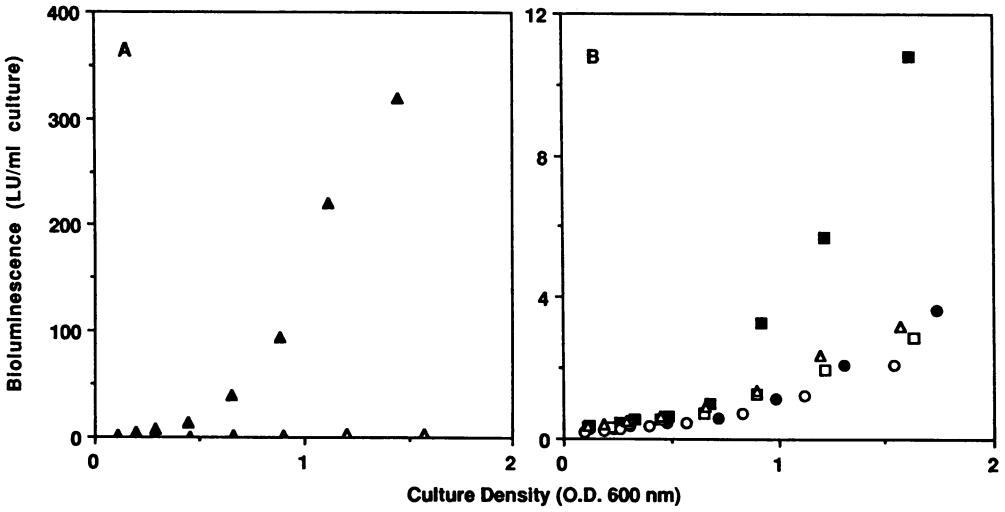

FIG. 6. Effects of two point mutations within the operator on induction. Cultures of *E. coli* TB1 bearing pJHD500 (triangles), pJHD500-op3CT (circles), and pJHD500-op5GC (squares) were grown at 27°C in LB/carbenicillin. Solid symbols, cultures that contain autoinducer; open symbols, uninduced cultures. (*A*) "Wild-type" pJHD500 induction profile. (*B*) Induction profiles of the two mutants (with the uninduced pJHD500 data included for reference purposes; note the difference in scale). LU, light units.

DISCUSSION

The results of the experiments reported here demonstrate a functional role for the 20-bp palindrome, ACCTG-TAGGA|TCGTACAGGT, located within the regulatory region between *luxR* and *luxI* (16). High-level transcription from P_R required this palindrome, functional LuxR, and autoinducer. Deletion of sequences on the 5′ side of the palindrome up to and including the 5′ AC of the palindrome resulted in an ≈10-fold increased level of transcription from P_R in the absence of LuxR–autoinducer, but transcription from P_R of the modified plasmid remained responsive to LuxR–autoinducer. LuxR–autoinducer stimulation of P_R was effectively blocked by a single-base change, C → T, at position 3 of the palindrome and was greatly reduced by another mutation, G → C, at position 5 of the palindrome. Basal transcription was normal with both mutants as well as with the mutant in which the central 12 bp of the operator were deleted, demonstrating that the operator has no (or minimal) function in repressing transcription from P_R in the absence of LuxR–autoinducer. We suggest that the palindrome is the primary binding site from which the LuxR–autoinducer complex functions to stimulate transcription from P_R. Sequences upstream of the palindrome appear to function in cis, by an unknown mechanism, to repress transcription of P_R in the absence of LuxR–autoinducer, thereby maintaining low basal levels of bioluminescence expression.

The *lux* regulon of *V. fischeri* possesses many features that are common to those of well-studied *E. coli* regulatory units, but its primary mechanism is a unique variation of positive feedback control. The *lux* regulon consists of two closely linked operons transcribed divergently from a common regulatory control region and governed by a transcriptional activator protein. The *araC–BAD*, *malT–PQ*, and *asnC–A* regulons of *E. coli* and the *cI–cro* regulon of phage λ have similar configurations. Of these four systems, only *ara* and *cI–cro* have been studied extensively.

The *ara* and *mal* systems have the greatest apparent similarity to *lux*. All three have the genes for their activator proteins isolated on one operon that is activated by the cAMP-binding protein–cAMP system. The similarity between *lux* and *ara* also includes autogenous regulation of the activator proteins, LuxR and AraC. Schleif and coworkers (26) have shown protein-mediated DNA looping to be a factor in the repression of uninduced transcription in the *araBAD* operon. DNA looping has also been invoked in the well-known *E. coli lac* operon (27). The results that we present here demonstrate the presence of sequences upstream of the primary operator that repress transcription from P_R, but it is uncertain at this time if looping is involved in the *lux* system.

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